TECHNIQUE FOR SUPRAVITAL STAINING

Note: It is very important to have true vital dyes. In this country, at present, the National Aniline and Chemical Company, Inc., New York City, N. Y., sells a vital neutral red and a vital Janus green (Diazine Green), prepared under the direction of Doctor H. J. Conn, Chairman of the Commission on Standardization of Biological Stains, Geneva, N. Y. Every sample is tested in my laboratory (Doctor Florence R. Sabin) before it is offered for sale.

The technique consists of getting a thin, evenly spread film of two dyes on a slide and then making a preparation of fresh blood on this slide. The two dyes become dissolved in the plasma and stain the cells differentially. The amount of the dye must be quite accurately measured. If there is too much, the cells are killed and dead cells in this technique cannot be discriminated. To get the dry dye on the slide, the solvent used is absolute alcohol which then is evaporated off. The alcohol must be entirely free of acid; the absolute alcohol, bought commercially, will not do. It must be distilled from lime in order to remove the last traces of acid.

I. Neutral red. It is convenient to have as a stock solution a saturated solution of neutral red in absolute alcohol. When procuring the neutral red, one must specify "for vital staining". A saturated solution is 0.25 per cent and is made by dissolving 125 mgms. of the dye in 50 cc. of the alcohol. This solution is stable.

It is far too strong for staining cells. To make the dilute solution, add 1.1 cc. of the saturated solution to 10 cc. of the absolute alcohol.

II. Janus green. The Janus green solution is made by dissolving 125 mgms. of the dye in 62.5 cc. of alcohol. This makes a saturated solution (0.20 per cent).

III. Mixed neutral red and Janus green. To make the solution which is actually used on the slides, take 3 cc. of the dilute neutral red and to it add 2 drops of the saturated Janus green solution. Have the slides thoroughly clean and free from grease, by the usual methods. Flood the slide with the stain and drain the excess immediately back into the bottle. Then let the slide dry in warm air. This is conveniently arranged by working very close to a lighted Bunsen burner. There should be no streaks whatever of the stain on the slide. The excess can be removed from the edges by placing the edges against a piece of cheesecloth. After the slides are once made, they keep almost indefinitely. In making the fresh preparations of blood, it is important to seal the edges of the coverslips with a vaseline of high melting point. We study our preparations in a warm-box regulated to 37° C. and if one is interested in motility of cells, it is necessary to have the right temperature. The supravital differential counts can, however, readily be made at room temperature and the cells are much less damaged by cold than by heat.

THE STAINING OF BLOOD CELLS WITH THE SUPRAVITAL TECHNIQUE

1. Granulocytes. The neutrophilic granules stain rather faintly with a salmon red color. You will notice if any myelocytes appear in the circulating blood that the staining of the granules is more intense than that of the mature leucocyte. The myelocytes also contain mitochondria which will stain green; mitochondria diminish as the cells mature, so the average leucocyte shows only a very few mitochondria. The granules of the basophilic leucocytes have a very bright red stain, which is the acid reaction of the dye. The large, usually ovoid, eosinophilic granules, on the other hand, stain either yellow or orange, which is the color of neutral red in alkaline solution. Besides the granules, the leucocytes, especially the neutrophilic type, have vacuoles if they have phagocytized material and the fluid in these vacuoles stains red with neutral red.

2. Lymphocytes. The lymphocytes are distinctive on account of their large content of mitochondria. They tend to be in the form of rods. The lymphocytes occur in three sizes: small, intermediate, and large, but perhaps it is only important to separate the large ones from the other two. The younger the cell, the more the mitochondria, and the very young lymphocytes show also a rather yellow tone in the cytoplasm which is due to the high content of basophilic substance. This is brought out better in fixed films. The lymphocytes also may have a few vacuoles staining in neutral red. Both the mitochondria and the vacuoles

tend to be in the endoplasm rather close to the nucleus. The characteristic pattern of the chromatin of the lymphocytic nuclei shows in the living cell.

3. Monocytes. Monocytes are characterized by delicate surface film in contrast to the sharp and dense borders of the lymphocytes. Monocytes are phagocytic cells and thus have many vacuoles, small or large, which stain with the neutral tone of neutral red. These vacuoles are often in the form of a rosette, especially in lower animals and in diseased states in man. Besides the vacuoles that stain in neutral red, there are very large numbers of tiny mitochondria which stain green. The chromatin network in the nucleus of the living monocyte is finer than that in the nucleus of the lymphocyte.

4. Red blood cells. You will notice in the supravital preparations that many of the red cells show a little reaction to the neutral red. This is reticulation but the dilution of the neutral red used for studying white cells is too great to include all the reticulocytes. If the stain is made concentrated enough for bringing out all the reticulocytes, it will kill the white cells.

5. Macrophages or clasmatocytes react to the vital neutral red; their vacuoles vary in size and while each vacuole stains uniformly, the different vacuoles show the full range of shades of neutral red from yellow to brilliant scarlet.

6. Application to pleural and ascitic fluids, and to biopsy material. The method can be applied to scrapings from organs and to the cells of pleural, peritoneal, or cerebrospinal fluids, as well as to various exudates.